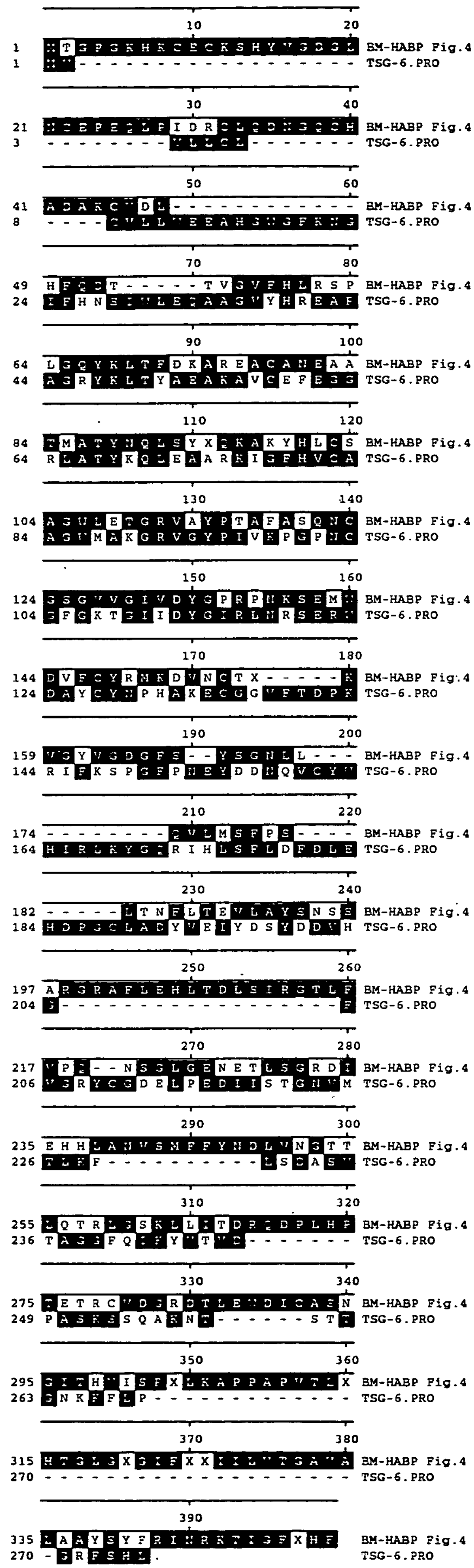


Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Sequence pair distances of Untitled, using J. Hein method with PAM250 residue weight table.

Percent Identity

Divergence

	1	2	
1		31.6	1
2	100.0		2
	1	2	

BM-HABP Fig.4.PRO

TSG-6.PRO

NCBI Blast 2 Sequences results

PubMed Entrez BLAST OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTP 2.1.2 [Oct-19-2000]

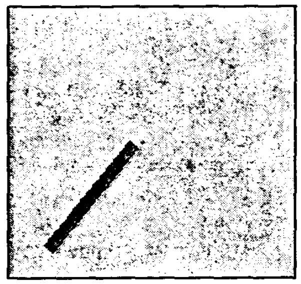
Matrix BLOSUM62 gap open: 11 gap extension: 1

x_dropoff: 50 expect: 10.000(wordsize: 3 Filter ☑ Align

Sequence 1 lcl|seq_1 **Length** 353 (1 .. 353)

Sequence 2 lcl|seq_2 **Length** 275 (1 .. 275)

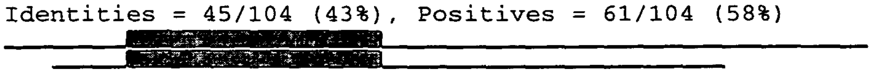
2



1

NOTE:The statistics (bitscore and expect value) is calculated based on the size of nr database

```
Score =  107 bits (264), Expect = 5e-21
Identities = 45/104 (43%), Positives = 61/104 (58%)
```



```
Query: 52   DTTVGVFHLRSPLGQYKLTFDKAREACANEAATMATYNQLSYXQKAKYHLCSAGWLETGR 111
            +    GV+H   +   G+YKLT+ +A+   C   E     +ATY QL     +K   +H+C+AGW+    GR
Sbjct: 32   EQAAGVYHREARAGRYKLTYAEAKAVCEFEGGRLATYKQLEAARKIGFHVCAAGWMAKGR 91

Query: 112  VAYPTAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYRMKDVNC 155
            V YP        NCG G   GI+DYG R N+SE WD +CY        C
Sbjct: 92   VGYPIVKPGPNCGFGKTGIIDYGIRLNRSERWDAYCYNPHAKEC 135

CPU time:     0.15 user secs.       0.03 sys. secs         0.18 total secs.

Gapped
Lambda     K      H
   0.321    0.138    0.429

Gapped
Lambda     K      H
   0.270   0.0470    0.230


Matrix: BLOSUM62
Gap Penalties: Existence: 11, Extension: 1
Number of Hits to DB: 748
Number of Sequences: 0
Number of extensions: 55
Number of successful extensions: 1
Number of sequences better than 10.0: 1
Number of HSP's better than 10.0 without gapping: 1
Number of HSP's successfully gapped in prelim test: 0
Number of HSP's that attempted gapping in prelim test: 0
Number of HSP's gapped (non-prelim): 1
```

```
length of query: 353
length of database: 3,171,650,076
effective HSP length: 58
effective length of query: 295
effective length of database: 3,171,650,018
effective search space: 935636755310
effective search space used: 935636755310
T: 9
A: 40
X1: 16 ( 7.4 bits)
X2: 128 (49.9 bits)
X3: 128 (49.9 bits)
S1: 41 (21.9 bits)
S2: 83 (36.7 bits)
```

EXHIBIT C

```
  56 GVFHLRSPLGQYKLTFDKAREACANEAATMATYNQLSYXQKAKYHLCSAGWLETGRVAYP -SEQ ID NO:11
     |||||||||||||||||||||||||||||||||||||| ||||||||||||||||||||
1063 GVFHLRSPLGQYKLTFDKAREACANEAATMATYNQLSYAQKAKYHLCSAGWLETGRVAYP -human HARE

 115 TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR    149
     ||||||||||||||||||||||||||||||||||
1122 TAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCYR   1156
```

CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 1

EDITORIAL BOARD

Frederick M. Ausubel
Massachusetts General Hospital & Harvard Medical School

Roger Brent
Massachusetts General Hospital & Harvard Medical School

Robert E. Kingston
Massachusetts General Hospital & Harvard Medical School

David D. Moore
Massachusetts General Hospital & Harvard Medical School

J.G. Seidman
Harvard Medical School

John A. Smith
University of Alabama

Kevin Struhl
Harvard Medical School

GUEST EDITORS

Lisa M. Albright
DNA Sequencing

Donald M. Coen
Harvard Medical School
Polymerase Chain Reaction

Ajit Varki
University of California San Diego
Glycoproteins

SERIES EDITOR

Virginia Benson Chanda

John Wiley & Sons, Inc.

CORE 13 (S36)

While the authors, editors, and publisher believe that the specification and usage of reagents, equipment, and devices, as set forth in this book, are in accord with current recommendations and practice at the time of publication, they accept no legal responsibility for any errors or omissions, and make no warranty, express or implied, with respect to material contained herein. In view of ongoing research, equipment modifications, changes in governmental regulations, and the constant flow of information relating to the use of experimental reagents, equipment, and devices, the reader is urged to review and evaluate the information provided in the package insert or instructions for each chemical, piece of equipment, reagent, or device for, among other things, any changes in the instructions or indication of usage and for added warnings and precautions. This is particularly important in regard to new or infrequently employed chemicals or experimental reagents.

Library of Congress Cataloging in Publication Data:

Current protocols in molecular biology. 3 vols.
I. Molecular biology—Technique. 2. Molecular biology—Laboratory manuals. I. Ausubel, Frederick M.

QH506.C87 1987 574.8'8'028 87-21033
ISBN 0-471-50338-X

Printed in the United States of America

20 19 18 17 16 15 14 13

1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

If signal is still seen after autoradiography, rewash using harsher conditions.

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.

REAGENTS AND SOLUTIONS

Aqueous prehybridization/hybridization (APH) solution

5× SSC (APPENDIX 2)
5× Denhardt solution (APPENDIX 2)
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Denatured salmon sperm DNA

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at −20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

Formamide prehybridization/hybridization (FPH) solution

5× SSC (APPENDIX 2)
5× Denhardt solution (APPENDIX 2)
50% (w/v) formamide
1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).

Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.

CAUTION: *Formamide is a teratogen. Handle with care.*

Labeling buffer

200 mM Tris-Cl, pH 7.5
30 mM $MgCl_2$
10 mM spermidine

Mild stripping solution

5 mM Tris-Cl, pH 8.0
2 mM EDTA
0.1× Denhardt solution (APPENDIX 2)

SDS electrophoresis buffer, 5×

15.1 g Tris base
72.0 g glycine
5.0 g SDS
H_2O to 1000 ml
Dilute to 1× or 2× for working solution, as appropriate

Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).

SED (standard enzyme diluent)

20 mM Tris·Cl, pH 7.5
500 µg/ml bovine serum albumin (Pentax Fraction V)
10 mM 2-mercaptoethanol
Store up to 1 month at 4°C

Sodium acetate, 3 M

Dissolve 408 g sodium acetate·$3H_2O$ in 800 ml H_2O
Add H_2O to 1 liter
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

Sodium acetate buffer, 0.1 M

Solution A: 11.55 ml glacial acetic acid/liter (0.2 M).
Solution B: 27.2 g sodium acetate ($NaC_2H_3O_2 \cdot 3H_2O$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 100 ml. (See Potassium acetate buffer recipe for further details.)

Sodium phosphate buffer, 0.1 M

Solution A: 27.6 g $NaH_2PO_4 \cdot H_2O$ per liter (0.2 M).
Solution B: 53.65 g $Na_2HPO_4 \cdot 7H_2O$ per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H_2O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

SSC (sodium chloride/sodium citrate), 20×

3 M NaCl (175 g/liter)
0.3 M Na_3citrate·$2H_2O$ (88 g/liter)
Adjust pH to 7.0 with 1 M HCl

STE buffer

10 mM Tris·Cl, pH 7.5
10 mM NaCl
1 mM EDTA, pH 8.0

TAE (Tris/acetate/EDTA) electrophoresis buffer

50× stock solution:
242 g Tris base
57.1 ml glacial acetic acid
37.2 g $Na_2EDTA \cdot 2H_2O$
H_2O to 1 liter

Working solution, pH ~8.5:
40 mM Tris·acetate
2 mM $Na_2EDTA \cdot 2H_2O$

TBE (Tris/borate/EDTA) electrophoresis buffer

10× stock solution, 1 liter:
108 g Tris base (890 mM)
55 g boric acid (890 mM)
40 ml 0.5 M EDTA, pH 8.0 (20 mM)